Association of nerve growth factor and vascular endothelial growth factor A with psychometric measurements of opiate dependence: results of a pilot study in patients participating in a structured diamorphine maintenance program

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Abstract: Preclinical study results suggest that neurotrophic peptides like nerve growth factor (NGF) and vascular endothelial growth factor A (VEGF-A) may be associated with symptoms of addictive behavior like withdrawal symptoms and rewarding effects. We investigated alterations in NGF and VEGF-A serum levels in opiate-dependent patients (25 male patients), who received diamorphine (DAM, heroin) treatment within a structured opiate maintenance program, and compared the results with the NGF and VEGF-A serum levels of healthy controls (23 male controls). NGF and VEGF-A serum levels were assessed before and after DAM administration twice a day (in the morning (16 h after last application–t1) and in the afternoon (7 h after last application–t3)) in order to detect a possible immediate or summative (in the afternoon) heroin effect on these two neuropeptides. Moreover, we investigated possible associations between the serum levels of these neurotrophic growth factors and psychometric dimensions of addictive behavior, e.g. craving, withdrawal, depression. Whereas there was no direct effect of DAM application on the serum levels of both neurotrophic growth factors neither in the morning nor in the afternoon, the NGF serum levels of the patient group were found to be significantly increased at all four time points of investigation compared with the healthy controls. In contrast, VEGF-A serum levels did not differ significantly in the patient and control groups. We found a significant positive association between the NGF serum levels and several items of the short opiate withdrawal scale as well as a negative association between self-reported mood (measured by visual analogue scale) and mood before heroin application (in the morning as in the afternoon). Moreover, we found a significant positive association between the NGF serum levels (t1 and t3) and the self-reported craving for methadone. In contrast, we found a negative association between the VEGF-A serum levels and avoidance, anxiety, suicide intentions of the SCL-90 as well as a positive association between the VEGF-A serum levels and the subscales of the heroin craving questionnaire measuring the rewarding effects of heroin. In conclusion, the results of this pilot study show that there might be an association between symptoms of opiate dependence and withdrawal and serum levels of VEGF-A and NGF.

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Association of Nerve Growth Factor and Vascular Endothelial Growth Factor A with Psychometric Measurements of Opiate Dependence: Results of a Pilot Study in Patients Participating in a Structured Diamorphine Maintenance Program

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Key Words

Opiate dependence · Opiate maintenance · Vascular endothelial growth factor A · Nerve growth factor · Neurotrophic growth factors · Craving

Abstract

Preclinical study results suggest that neurotrophic peptides like nerve growth factor (NGF) and vascular endothelial growth factor A (VEGF-A) may be associated with symptoms of addictive behavior like withdrawal symptoms and rewarding effects. We investigated alterations in NGF and VEGF-A serum levels in opiate-dependent patients (25 male patients), who received diamorphine (DAM, heroin) treatment within a structured opiate maintenance program, and compared the results with the NGF and VEGF-A serum levels of healthy controls (23 male controls). NGF and VEGF-A serum levels were assessed before and after DAM administration twice a day (in the morning (16 h after last application – t1) and in the afternoon (7 h after last application – t3)) in order to detect a possible immediate or summative (in the afternoon) heroin effect on these two neuropeptides. Moreover, we investigated possible associations between the serum levels of these neurotrophic growth factors and psychometric dimensions of addictive behavior, e.g. craving, withdrawal, depression. Whereas there was no direct effect of DAM application on the serum levels of both neurotrophic growth factors neither in the morning nor in the afternoon, the NGF serum levels of the patient group were found to be significantly increased at all four time points of investigation compared with the healthy controls. In contrast, VEGF-A serum levels did not differ significantly in the patient and control groups. We found a significant positive association between the NGF serum levels and several items of the short opiate withdrawal scale as well as a negative association between self-reported mood (measured by visual analogue scale) and mood before heroin application (in the morning as in the afternoon). Moreover, we found a significant positive asso-

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ciation between the NGF serum levels (t1 and t3) and the self-reported craving for methadone. In contrast, we found a negative association between the VEGF-A serum levels and avoidance, anxiety, suicide intentions of the SCL-90 as well as a positive association between the VEGF-A serum levels and the subscales of the heroin craving questionnaire measuring the rewarding effects of heroin. In conclusion, the results of this pilot study show that there might be an association between symptoms of opiate dependence and withdrawal and serum levels of VEGF-A and NGF.

Introduction

Opiates, in particular heroin, are known for their high addictive potential which may be tracked back, on the neurobiological level, to the activation of mesolimbic and mesocortical dopamine neurons as well as to subsequent alterations in midbrain dopamine release [1, 2]. On the bio-behavioral level, use of heroin typically results in a period of euphoria and tension relief [3], which is followed by a period of dysphoria and increase in tension when heroin's stimulating effects are fading [4]. Dysphoria and increase in tension – as bodily symptoms like lacrimation, headache, muscle cramps and increase of blood pressure – are typical symptoms of the opiate withdrawal syndrome. Heroin's short half-life of approximately 4–6 h causes the recurrence of typical opiate withdrawal symptoms in opiate-dependent patients, which is one reason for the need of recurrent drug consume and its social implications [5].

Actual research results confirm that neurotrophic growth factors like nerve growth factor (NGF) are involved in the regulation of midbrain dopamine release [6]. Moreover, clinical studies suggest that neurotrophic growth factors may be associated with mood symptoms like antidepressant effects (vascular endothelial growth factor A, VEGF-A) or stress (NGF). For example, an increase in circulating NGF levels has been observed in combating male mice [7] and, in particular, higher NGF serum levels have been observed in defeated, subordinated compared to dominant mice supporting the hypothesis of an association between NGF serum levels and stressful life events [8]. Consistently, in parachuters' NGF serum levels were reported to increase just before their first jump [9]. These and further study results [10] suggest that alterations of NGF may be part of the neurobiological stress cascade. This hypothesis is further strengthened by some preclinical study results that report about an association between cortisol serum levels and NGF serum levels [11]. Therefore, one could speculate that there might be an association between withdrawal symptoms and NGF serum levels as well. Consistent with such a hypothesis, we found increased NGF serum levels in alcohol-dependent patients, which decreased during alcohol withdrawal [12, 13].

Although the expression of the angiogenic VEGF-A has been shown to be associated with the expression of NGF [14], clinical study results suggest different associations between VEGF-A and mood compared to NGF's effects. In particular, the results given point towards an association between VEGF-A and antidepressant effects [15] and that polymorphisms of the VEGF-A gene may be relevant for the therapeutic outcome of antidepressant treatment [16].

With respect to such study results, we aimed to investigate putative associations between psychometric measurements of opiate reward and opiate withdrawal in opiate-dependent patients in a structured diamorphine (DAM) maintenance program.

Subjects and Methods

We investigated 25 opiate-dependent male patients (see table 1 for details) recruited from the Heroin Prescription Center of the Psychiatric Hospital of the University of Basel [17]. All patients fulfilled the diagnostic criteria of opiate dependence according to ICD-10 and DSM-IV, and had participated in a DAM maintenance program for at least 2 months before participating in the study. All patients received individual doses of injectable DAM (administered intravenously or intramuscularly) twice a day. Additionally, 8 patients received oral methadone (mean 22.85 mg, SD 14.1 mg) and 7 patients oral DAM treatment (mean 300.0 mg, SD 173.21 mg). Patients suffering from axis-I diagnoses other than opiate dependence and substance abuse were excluded from the study, as were patients showing positive breath alcohol concentrations. The study adhered to the Declaration of Helsinki and was approved by the Ethics Committee of the University of Basel. All patients gave written informed consent.

General Procedures

All patients underwent a 1-day experiment consisting of a morning and an afternoon testing session at the clinic. The procedures were scheduled to guarantee that patients could administer their DAM injections at the regular time, with an interval of 16 ± 1 h since the last DAM injection for the morning and a 7-hour interval for the afternoon session. The individual day of testing was randomly allocated during a 2-week study period, except for Saturdays which were reserved for those working. On admission, breath-alcohol content was measured using a breathalyzer (Dräger Alcotest 7410 Plus, Germany) in order to verify alcohol abstinence. Participants then chose a sealed envelope containing five sets of self-report measures. After completing

the initial set of self-report instruments, the first blood sampling occurred. 5 min later, patients self-injected their prescribed dose of DAM as usual. 5 min after injection, patients were administered the second set of measures. 45 min thereafter the second blood sampling was performed. Patients then completed the next set of instruments (administered only once during the experiment).

In the afternoon, the testing followed the same protocol, except that patients had to deliver a urine sample at the end of testing and received their monetary compensation.

Laboratory Analyses

Urine samples were analyzed by the Clinical Chemical Laboratory of the University Hospital Basel for the presence of alcohol, amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, ecstasy, methadone and opiates using enzyme immunoassays.

NGF and VEGF-A serum levels were investigated directly before and 45 min after regular injection of DAM in the morning (time frame of last injection 16 h (t1 and t2)) and in the afternoon (7 h after the morning injection (t3 and t4)). NGF and VEGF-A were assessed using the DuoSet enzyme-linked immunosorbent assay (ELISA) Development System (DY256, DY293 B; R&D Systems, Wiesbaden-Nordenstadt, Germany). All the assays were performed according to the manufacturer's instructions.

NGF and VEGF-A serum levels obtained in the opiate-dependent patients were compared to the NGF and VEGF-A serum levels of healthy controls (23 healthy male controls, mean age 27.90 years, SD 3.35 years). Controls were screened for substance abuse as well as for axis-I diagnoses using a structured interview. Controls were negative for axis-I diagnoses according to ICD-10 or DSM-IV. All controls and patients investigated were Caucasians.

Patient-Rated Measurements

All patients completed a battery of standardized self-completion instruments presented in paper-and-pencil format. The order of instruments, administered twice pre- and post-injection, was counterbalanced across participants and design, and included two visual analogue scales (VAS) – the Heroin Craving Questionnaire [18] and the Short Opiate Withdrawal Scale [19]. Additionally, patients completed once during testing a set of questionnaires in random order, including the Substance Use History Form, the Center of Epidemiologic Studies – Depression Scale [20] and the Symptom Checklist-27 [21].

Visual Analogue Scales. Patients rated their current state of heroin craving on a 100-mm horizontal line labeled from 'no craving at all' to 'very strong craving'. Furthermore, participants responded to a 100-mm mood VAS ('How is your general mood right now?') anchored with a sad face at the left end and a happy face at the right end.

Heroin Craving Questionnaire (HCQ). This multidimensional questionnaire presents 45 items rated on a 7-point scale from 'strongly disagree' to 'strongly agree'. Scoring yields 5 theory-derived subscales, measuring different aspects of craving.

Short Opiate Withdrawal Scale (SOWS). This 10-item scale assesses symptoms of opioid withdrawal on a 4-point intensity scale from 'none' to 'severe'.

Symptom Checklist-27 (SCL-27). This modification of the German Symptom Checklist-90-R screens for psychiatric symptoms on six subscales. Items are rated on a 5-point Likert scale, ranging from 'not at all' to 'extremely'.

Table 1. Demographic data of the heroin-dependent patients and the healthy controls

	Heroin-addicted patients (n = 28)	Healthy controls (n = 43)
Mean age, years	41.25 ± 6.4	24.93 ± 4.21
Range	28 – 54	18 – 37
Age of first use, years	19.86 ± 5.35	NA
Range	13 – 35	
Average daily dose of DAM, mg	323.21 ± 122.26	NA
SOWS score		
t1	19.07 ± 5.93	NA
t2	12.00 ± 2.76	NA
t3	12.53 ± 2.70	NA
t4	12.91 ± 1.91	NA
Mean HCQ		
t1	5.28 ± 0.79	NA
t2	3.56 ± 1.12	NA
t3	4.95 ± 0.88	NA
t4	3.55 ± 1.21	NA
SCL – total score	18.82 ± 15.13	NA
NGF		
t1	237.09 ± 250.5	109.14 ± 145.35
t2	238.51 ± 260.81	
t3	262.71 ± 293.79	
t4	245.41 ± 314.20	
VEGF-A		
t1	62.59 ± 42.18	49.51 ± 42.33
t2	66.15 ± 50.44	
t3	65.95 ± 41.82	
t4	62.40 ± 44.08	

Statistical Analyses

VEGF-A serum levels were normally distributed according to the Kolmogorov-Smirnov test. NGF serum levels were ln-transformed in order to reach normal distribution. Correlations between the VEGF-A and the ln-transformed NGF serum levels and the psychometric dimensions of heroin craving were calculated using Pearson's correlation coefficient. Differences between the VEGF-A and the ln-transformed NGF serum levels of the opiate-dependent patients and the healthy control group were calculated using the t test for independent samples. Alterations of the ln-transformed NGF and VEGF-A serum levels before and after the injection of DAM were assessed using the t test for dependent samples. The data was analyzed using PASW Statistics 18.0 and Graph Pad Prism™ 5.0 (Graph Pad Software Inc., San Diego, Calif., USA).

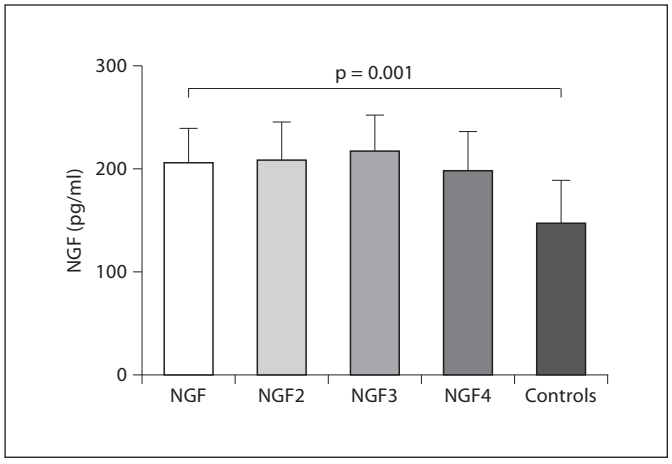

Fig. 1. Means and SEM of NGF serum levels before (NGF, NGF3) and after (NGF2, NGF4) the scheduled injection of DAM in opiate-dependent patients compared to healthy controls.

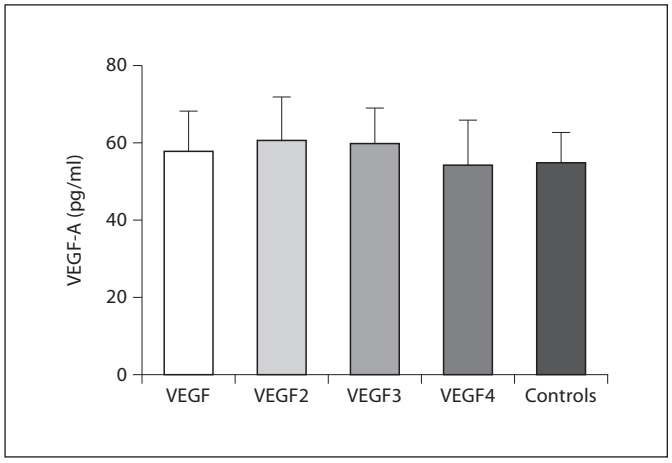

Fig. 2. Means and SEM of the VEGF-A serum levels before (VEGF, VEGF3) and after (VEGF2, VEGF4) the scheduled injection of DAM in opiate-dependent patients compared to healthy controls. VEGF-A serum levels found in the patient group did not differ significantly from the VEGF-A serum levels obtained from healthy controls.

Results

VEGF-A serum levels were not associated with the duration of opiate dependence, age, or dosage of DAM or methadone, respectively (data not shown). The ln-transformed NGF serum levels showed a trend towards a negative association with the duration of opiate dependence ($r = -0.388$, $p = 0.056$). The ln-transformed NGF serum levels were not associated with the age of the patients or the dosage of DAM injected (data not shown). There was a significant association between the dosage of methadone and NGF serum levels at each time point of investigation (t1: $r = 0.434$, $p = 0.021$; t2: $r = 0.627$, $p < 0.001$; t3: $r = 0.422$, $p = 0.028$; t4: $r = 0.442$, $p = 0.021$).

(a) Comparison of the NGF and VEGF-A serum levels of the patients and the control group: NGF serum levels were significantly increased in the opiate-dependent patients before and after the application of DAM in the morning as in the afternoon ($T = 2.483$, $p = 0.017$ (t1); $T = 2.394$, $p = 0.022$ (t2); $T = 2.569$, $p = 0.015$ (t3); $T = 2.150$, $p = 0.039$ (t4)). VEGF-A serum levels were not significantly increased compared to the serum levels of the healthy controls either before and after DAM injection in the morning or in the afternoon (see table 1 for details). There was no significant difference in the NGF or VEGF-A serum levels of patients receiving additional methadone compared with patients receiving a monotherapy with DAM (data not shown).

(b) Alterations of the NGF and VEGF-A serum levels after application of DAM: Comparison of the NGF and VEGF-A serum levels before and after injection of the regular dosage of DAM showed no significant differences in the morning or in the afternoon (see table 1 for details). NGF and VEGF-A serum levels were not associated with each other (fig. 1, 2).

(c) Association between psychometric dimensions of opiate dependence and the NGF and VEGF-A serum levels.

Nerve Growth Factor

NGF serum levels were significantly associated with nausea ($r = 0.545$, $p = 0.003$), lacrimation and running nose ($r = 0.378$, $p = 0.047$) at t1, with nausea ($r = 0.510$, $p = 0.006$), gastrospasm ($r = 0.381$, $p = 0.50$), sensations of heat and cold ($r = 0.469$, $p = 0.040$), muscle spasm ($r = 0.543$, $p = 0.003$), lacrimation and running nose ($r = 0.722$, $p < 0.001$) at t2, lacrimation and running nose ($r = 0.626$, $p < 0.001$) at t3 and ($r = 0.515$, $p < 0.001$) at t4. Moreover, there was a negative correlation between the VAS measurements of mood and NGF serum levels on t1 ($r = -0.537$, $p = 0.003$) and t3 ($r = -0.356$, $p = 0.068$).

NGF serum levels on t1 ($r = 0.725$, $p < 0.001$) and t3 ($r = 0.385$, $p = 0.047$) were significantly associated with craving for methadone measured by VAS (there was no association with VAS measuring craving for heroin, alcohol and cocaine). Associations between opiate withdrawal and NGF serum levels as well as between mood

and NGF serum levels were found in patients with and without additional methadone treatment (data not shown).

Vascular Endothelial Growth Factor A

There was a negative association between the VEGF-A serum levels and avoidance ($r = -0.719$, $p = 0.019$), anxiety ($r = -0.718$, $p = 0.019$), suicide intentions ($r = -0.704$, $p = 0.023$) of the SCL-90.

VEGF-A serum levels were significantly associated with the subscale measuring desire for heroin at t2 ($r = 0.712$, $p = 0.014$) and t4 ($r = 0.834$, $p = 0.003$), anticipation of heroin effects at t2 ($r = 0.701$, $p = 0.016$) and at t4 ($r = 0.785$, $p = 0.007$) and the intention of application of heroin at t2 ($r = 0.590$, $p = 0.056$) and t4 ($r = 0.681$, $p = 0.030$).

Comparison between psychometric dimensions between patients receiving additional methadone and patients being treated with a monotherapy of DAM.

Patients receiving additional methadone rated their actual mood worse at t1 ($t = -2.249$, $p = 0.033$) and t4 ($t4 = -4.992$, $p < 0.001$) than patients being treated with a monotherapy of DAM. Moreover, the methadone group showed a significant increase in SCL-90 point score ($t = 3.454$, $p = 0.002$). There were no further significant differences in the psychometric measurements of the two subgroups (data not shown).

Discussion

In this pilot study, we found increased serum levels of NGF in the opiate-dependent patients during DAM maintenance, whereas the VEGF-A serum levels were not significantly changed as compared to healthy controls. According to clinical study results describing a negative association between VEGF-A serum levels and depression, we found a negative association between negative emotions like anxiety, avoidance and suicide intentions and the VEGF-A serum levels in the opiate-dependent patients. Moreover, we found a positive association between some aspects of heroin craving, like the desire for and the anticipation of desirable heroin effects, and the VEGF-A serum levels measured directly after application of DAM in the morning (t2) and in the afternoon (t4). This observation is consistent with the putative association between VEGF-A and depression reported before [15, 16, 22]. These results suggest that the angiogenic factor VEGF-A may be directly involved in the perception and/or constitution of positive DAM effects, although the neurobiological mechanisms that may explain this association are not fully understood. Recent research indicates that there is not only an association between VEGF-A and hippocampal growth but also an association between VEGF-A and signaling pathways that are related to anxiety and depression [23]. Therefore, VEGF-A may not only take influence on the development of addictive behavior by procession of hippocampal neuronal cell growth necessary for the consolidation of memory [24], but may also directly impact the consolidation of rewarding drug effects by long-term potentiation of synaptic strength. A putative impact of VEGF-A in long-term alterations of intersynaptic communication of signaling pathways related to anxiety and depression would explain that an association between psychometric dimensions of anxiety and depression are not only found in patients but also in healthy students [25], although the association observed in this study between the rewarding and relieving effects of heroin and the VEGF-A serum levels following application of DAM in the morning (t2) and in the afternoon (t3) may point towards neuroadaptive processes in the VEGF-A signaling system due to recurrent DAM (or heroin) application.

In contrast to the negative association between anxiety and VEGF-A serum levels, we found a positive association between the (increased) NGF serum levels and symptoms of opiate withdrawal measured by the SOWS. Consistent with this finding, we found a negative correlation between the VAS measuring the actual mood and NGF serum levels before application of DAM in the morning (t1) and in the afternoon (t3). Therefore, presuming an indicative function of NGF and stress as suggested by various studies [9, 11], the increased NGF serum levels in the patients participating in the structured DAM maintenance program may point towards a high level of stress most likely induced by recurrent opiate withdrawal symptoms based on the short half-life of DAM. This hypothesis is strengthened by the association between the NGF serum levels of the patient group and the craving for the methadone before application of DAM in the morning (t1) as in the afternoon (t3). This selective association between the NGF serum levels and methadone (and the lack of a similar association between the NGF serum levels and the craving for alcohol, cocaine or heroin) may be explained by the long half-life of methadone and its long-lasting prevention of opiate withdrawal symptoms.

In conclusion, both VEGF-A and NGF serum levels may be useful as potential biomarkers of opiate craving and withdrawal. Although this study has several limita-

tions which have to be addressed, we investigated peripheral VEGF-A and NGF serum levels, but until today it is not clear whether the peripheral blood levels of these neurotrophic growth factors are related to the intracerebral levels of these neuropeptides. Further, both growth factors are known to be associated with cytokine regulation [26, 27]. Therefore, inflammatory processes may have influenced the serum levels of both growth factors investigated in this study, which we did not control. Moreover, as is typical for opiate-dependent patients treated with DAM [28, 29], patients enrolled in this study were not only treated with DAM but also with further substances like methadone or antidepressants. Urine samples revealed that the patients also consumed further psychotrophic substances like benzodiazepines and amphetamines. Therefore, it cannot be ruled out that our results may be biased by this kind of 'comedication' typical for patients suffering from polytoxicomania [30].

Regarding the association between NGF and stress and the negative association between VEGF-A and anxiety and depression, our results fit well with former preclinical and clinical study results. Moreover, the associations found in this study are consistent with typical clinical symptoms of opiate dependence, for example the selectivity of the association between NGF serum levels and symptoms of opiate withdrawal at t1 and t3 of the investigation, which follow a period of abstinence of several hours, bolster the relevance of our findings.

In conclusion, our results point towards a potential association between peripheral serum levels of the neurotrophic growth factors VEGF-A and NGF and symptoms of opiate dependence in patients treated with DAM. Further studies will be necessary to validate the intriguing findings of our study and to enhance the understanding of the relevance of neurotrophic growth factors for the opiate dependence.

Disclosure Statement

The authors have no conflicts of interest to disclose.

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